

Plasma Scopoletin Levels after a Single Dose Oral Administration in Rabbits

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ABSTRACT

The pharmacokinetics of orally administered scopoletin were assessed in eight rabbits. The plasma concentrations of scopoletin after a single dose (50, 100 and 250 mg/kg, p.o.) were determined by HPLC. Pharmacokinetic parameters were calculated based upon a two-compartment open model. Peak plasma scopoletin concentrations ($C_{\max}$) of 0.506 ± 0.017 , 0.678 ± 0.026 and 1.488 ± 0.065 $\mu\text{g/mL}$ were reached about 40 minutes after administration of 50, 100 and 250 mg/kg scopoletin, respectively. The elimination half-lives ($t_{1/2\beta}$) were 78.8 ± 4.0 , 79.9 ± 5.8 and 80.5 ± 7.6 minutes and the area under the plasma level-time curve (AUC) were 67.7 ± 1.4 , 85.1 ± 2.6 and 173.8 ± 15.4 $\mu\text{g}\cdot\text{min/mL}$, respectively. The bioavailability (F) estimated from previous data and this experiment was about 0.19, 0.11 and 0.10, respectively. The results indicate that scopoletin exhibits dose-dependent pharmacokinetics after oral administration in the dose range of 50 to 250 mg/kg.

Key Words: Scopoletin; Oral; Pharmacokinetics

INTRODUCTION

Scopoletin (7-hydroxy-6-methoxycoumarin) is one of the main constituents in some traditional Chinese medicinal materials, including *Atropa belladonna* L. (Dinqiecao), *Angelica dahurica* BENTH et HOOK (Baizhi), *Angelica sinensis* DIELS (Danggui)¹ and *Lycium barbarum* L. (Gouqizi).² Scopoletin has been investigated as an antirheumatic remedy due to its anti-inflammatory effects.³ Scopoletin was about five times more potent than aspirin at inhibiting prostaglandin synthetase.⁴

The pharmacokinetics of scopoletin after intravenous administration in rabbits have

been described.⁵ Scopoletin exhibited non-linear pharmacokinetics over a 2-50 mg/kg dose range in rabbits. In the present study, the pharmacokinetic parameters of scopoletin were determined after oral administration of three different single doses in rabbits.

MATERIALS AND METHODS

Materials

Scopoletin was purchased from TCI (Tokyo Kasei, Japan), methylparaben was from Sigma (Saint Louis MO, USA), and methanol was from Ridel-de Haen (Hoechst, Germany). All other reagents and solvents were analytical grade.

Animal Studies

Eight New Zealand male white rabbits weighing about 2 to 3 kg were used in this study. These animals were housed at a temperature of 24 ± 1 °C with a light-dark cycle of 12-12 hr for at least one week before use. The rabbits were fasted overnight with free access to water before administration of scopoletin.

The oral scopoletin solution contained 10 mg/mL of scopoletin in aqueous 0.01 N NaOH. The washout period between each dose was at least one week. Scopoletin was given orally by a stomach tube at a single dose of 50, 100 or 250 mg/kg, respectively.

Blood Samples

Blood samples (1.25 ml) were drawn from the marginal ear vein of each rabbit at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300 and 360 minutes after drug administration. The blood samples were collected into heparinized tubes and were immediately centrifuged at 3,000 rpm for 10 min. The plasma was separated and stored at -30 °C until analysis.

HPLC Assay Method

The plasma scopoletin concentrations were determined by a HPLC method as described.⁵ Briefly, 200 µL of plasma was mixed with 800 µL of acetonitrile for deproteinization. 15 µL of internal standard solution (10 µg/mL of methylparaben in methanol) was added to each sample. The protein precipitate was separated by centrifugation. The supernatant was evaporated under nitrogen gas and the residue was reconstituted with 200 µL of the mobile phase (methanol-water 50:50 v/v). 50 µL of aliquot was injected onto the HPLC system (JASCO Model PU 980, Japan) for analysis. The validation samples were treated by the same process. The standard curve was linear over the concentration range of

0.01 to 100 $\mu\text{g/mL}$. The detection limit for scopoletin under these conditions was 10 ng/mL.

Data and Statistical Analysis

Plasma scopoletin concentrations versus time data were used for curve fitting by the computer program WinNonlin (WinNonlin Version 1.1, Scientific Consulting, Inc. USA), from which the pharmacokinetic parameters were estimated. The parameters were calculated from the following relationships:

$$\text{Cl} = F \cdot \text{Dose} / \text{AUC} \quad (1)$$

$$\alpha\beta = k_{10} \cdot k_{21} \quad (2)$$

$$\text{Cl} = k_{10} \cdot V_1 \quad (3)$$

$$V_{ss} = V_1 (1 + k_{12}/k_{21}) \quad (4)$$

All pharmacokinetic parameters are reported as mean $\pm$ SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) with the level of significance set at $p < 0.05$.

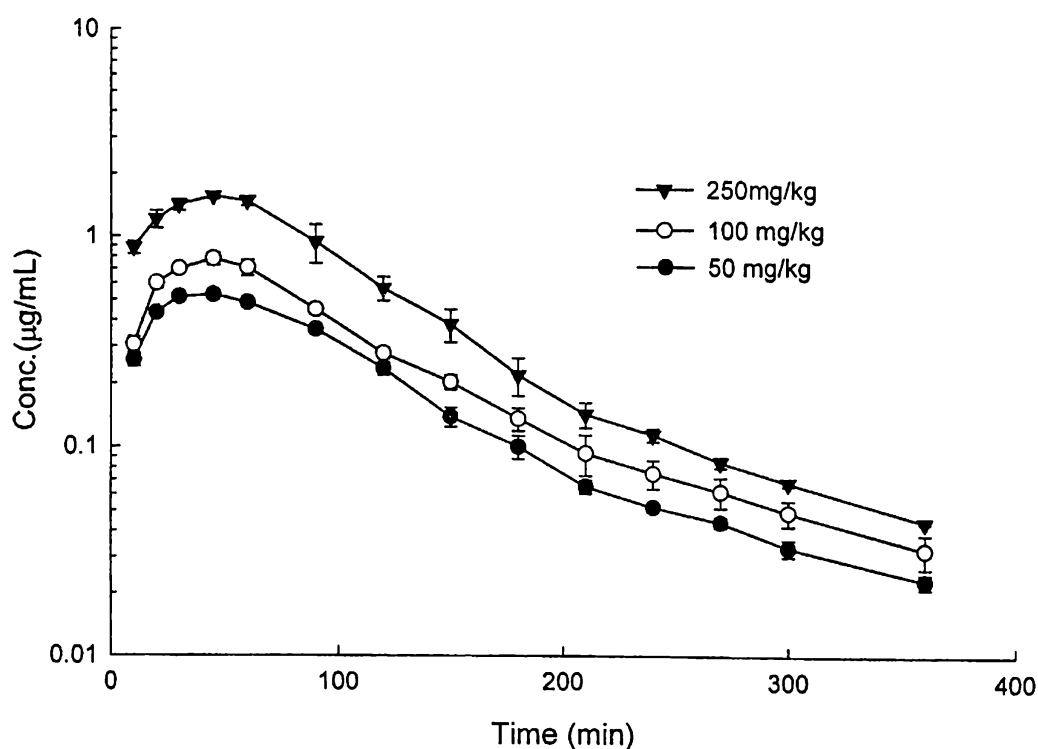


Fig. 1. Mean plasma concentration-time profiles of scopoletin after oral administration of 50, 100, or 250 mg/kg in eight rabbits. Each value indicates mean $\pm$ SD ($n = 8$)

RESULTS AND DISCUSSION

The mean plasma concentration-time profiles of scopoletin following oral administration of 50, 100 and 250 mg per kg scopoletin to rabbits are illustrated in Fig 1. Scopoletin was detectable in the plasma at 10 min after oral administration. Rapid absorption is obvious, with peak plasma concentration being obtained after about 40 min. No lag time was observed in the absorption of scopoletin in these eight rabbits. The mean peak concentration was 0.506 ± 0.017 , 0.678 ± 0.026 and 1.488 ± 0.065 $\mu\text{g/mL}$, respectively for the 50, 100 and 250 mg/kg oral doses.

When scopoletin was administered orally, the plasma concentration reached a peak and showed a biphasic decline. The computer program WinNonlin proposed a two compartment open model with individual plasma data after each dose. The following equation applies for a two compartment pharmacokinetic model after oral administration.

$$C_p (\mu\text{g/mL}) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-k_a t} \quad (5)$$

$$\text{Where } C = -(A + B)$$

The constants A, B and C are intercepts on the y axis for each exponential segment of the curve in equation (5) and α , β and k_a are the rate constants for distribution, elimination and absorption phases, respectively. Analysis of the data after oral administration of scopoletin at 50, 100 and 250 mg/kg doses yielded equations 6-8, respectively.

$$C_p = 3.102e^{-0.025t} + 0.472e^{-0.009t} - 3.574e^{-0.034t} \quad (6)$$

$$C_p = 4.894e^{-0.026t} + 0.491e^{-0.009t} - 5.385e^{-0.033t} \quad (7)$$

$$C_p = 11.057e^{-0.024t} + 0.740e^{-0.009t} - 11.797e^{-0.036t} \quad (8)$$

Table 1 shows the mean pharmacokinetic parameters for scopoletin after oral administration. The elimination half-life values (mean $\pm$ SD) after the administration of 50, 100 and 250 mg/kg doses of scopoletin were 78.8 ± 4.0 , 79.9 ± 5.8 and 80.5 ± 7.6 minutes, respectively. There was no statistically significant difference ($p > 0.05$) in the $t_{1/2\beta}$ values. Previously, we reported the pharmacokinetics of scopoletin after intravenous injection. The elimination half-lives were 74.3 ± 16.3 , 74.5 ± 12.8 and 72.0 ± 2.4 minutes for 2, 10 and 50 mg/kg intravenous (IV) bolus doses, respectively.⁵ The present study shows that the $t_{1/2\beta}$ values were similar after oral and IV administration of scopoletin.

The absorption half-lives (k_a -HL) were 20.7 ± 1.0 , 20.8 ± 0.7 and 19.6 ± 2.7 min, which corresponds to peak concentration times (t_{max}) of 39.9 ± 1.8 , 38.4 ± 1.0 and $36.8 \pm$

Table 1. Pharmacokinetic Parameters after Oral Administration of Scopoletin to Eight Rabbits

	50 mg/kg	100 mg/kg	250 mg/kg
A($\mu\text{g/mL}$) *	3.102 $\pm$ 1.103	4.894 $\pm$ 0.967	11.057 $\pm$ 6.548
B($\mu\text{g/mL}$)	0.472 $\pm$ 0.082	0.491 $\pm$ 0.086	0.740 $\pm$ 0.301
k _a (min ⁻¹)	0.034 $\pm$ 0.002	0.033 $\pm$ 0.001	0.036 $\pm$ 0.006
α (min ⁻¹)	0.025 $\pm$ 0.002	0.026 $\pm$ 0.001	0.024 $\pm$ 0.004
β (min ⁻¹)	0.009 $\pm$ 0.001	0.009 $\pm$ 0.001	0.009 $\pm$ 0.001
k ₁₀ (min ⁻¹) *	0.016 $\pm$ 0.001	0.017 $\pm$ 0.001	0.018 $\pm$ 0.003
k ₁₂ (min ⁻¹)	0.004 $\pm$ 0.001	0.004 $\pm$ 0.001	0.003 $\pm$ 0.001
k ₂₁ (min ⁻¹)*	0.014 $\pm$ 0.002	0.013 $\pm$ 0.001	0.011 $\pm$ 0.001
AUC($\mu\text{g}\cdot\text{min/mL}$) *	67.7 $\pm$ 1.4	85.1 $\pm$ 2.6	173.8 $\pm$ 15.4
K _a -HL(min)	20.7 $\pm$ 1.0	20.8 $\pm$ 0.7	19.6 $\pm$ 2.7
t _{1/2α} (min)	27.5 $\pm$ 2.4	27.2 $\pm$ 1.2	29.8 $\pm$ 5.4
t _{1/2β} (min)	78.8 $\pm$ 4.0	79.9 $\pm$ 5.8	80.5 $\pm$ 7.6
V ₁ /F (L) *	126.86 $\pm$ 10.66	189.73 $\pm$ 13.11	219.29 $\pm$ 38.63
Cl/F (L/min)*	1.99 $\pm$ 0.10	3.29 $\pm$ 0.17	3.99 $\pm$ 0.45
t _{max} (min) *	39.9 $\pm$ 1.8	38.4 $\pm$ 1.0	36.8 $\pm$ 2.9
C _{max} ($\mu\text{g/mL}$) *	0.506 $\pm$ 0.017	0.678 $\pm$ 0.026	1.488 $\pm$ 0.065

Data are expressed as mean $\pm$ SD (n = 8)

*Significantly different (p < 0.05) compared among 50, 100 and 250 mg/kg

2.9 min, respectively. The time to reach peak concentration became shorter when the oral dose was increased.

After a certain period following oral administration of scopoletin, the drug will equilibrate between various tissues and fluids, and net loss of drug from the central compartment to the tissue compartment will no longer occur. Once equilibrium is reached between the two compartments, the rate of drug loss from the blood stream is reduced. This period of more slowly declining blood level is the β phase. During the β phase, drug levels in both compartments will decline at the same rate, although the amount of drug in the two compartments may be different depending on the relative affinity of drug for various central and peripheral tissues. Based on a comparison of the ratio of intercompartment transfer rate constants, i.e. k₁₂ (0.004 $\pm$ 0.001, 0.004 $\pm$ 0.001 and 0.003 $\pm$ 0.001 min⁻¹) and k₂₁ (0.014 $\pm$ 0.002, 0.013 $\pm$ 0.001 and 0.013 $\pm$ 0.001 min⁻¹), the rate of transfer of scopoletin from the peripheral to the central compartment was about three times the rate of transfer from the central to the peripheral compartment. Hence, once steady state is reached, the

amount of scopoletin in the central compartment is greater than in the peripheral compartment. The value of k_{21} decreased as the dose increased as shown in Table 1. According to the relationship of $\alpha\beta = k_{10} \cdot k_{21}$, since the value of the elimination constant from the central compartment, k_{10} , increased as the dose increased, and there were no significant difference between the doses for the mean value of α and β , the value of k_{21} decreased as the dose increased. This suggested that the intrinsic rate of scopoletin loss from the central compartment increased at higher doses. Again, β is a complex rate constant obtained from the terminal elimination phase. In Table 1, the intrinsic rate constant for elimination of the drug from the central compartment (k_{10}) increased as the dose increased due to changes of k_{21} as related by $k_{10} = \alpha\beta/k_{21}$.

The mean AUC values of scopoletin after oral administration were 67.7 ± 1.4 , 85.1 ± 2.6 and 173.8 ± 15.4 $\mu\text{g} \cdot \text{min}/\text{mL}$, respectively, for 50, 100 and 250 mg/kg oral doses of scopoletin. In the previous study, we found that the AUC values and plasma levels of scopoletin were disproportionately increased with dose after IV administration, suggested that higher doses of scopoletin may result in nonlinear kinetics. However, there was no statistical difference between the volume of distribution at steady state (V_{ss}) and terminal half-life over the dose range of 2-10 mg/kg, showing that the pharmacokinetics of scopoletin following IV administration were linear over this dose range. Absolute bioavailability is estimated from the ratio of the AUC values obtained after both oral and IV administration of a drug. Since no IV data were measured in the present study, we assumed that the AUC following an IV dose of 10 mg/kg is linear. We thus estimated bioavailability values of 0.19 ± 0.01 , 0.11 ± 0.01 and 0.10 ± 0.01 for 50, 100 and 250 mg/kg oral doses, respectively. The bioavailability of scopoletin after oral administration appears to be decreased as the dose is increased.

The mean Cl/F values calculated by using AUC values from each dose were 1.99 ± 0.10 , 3.29 ± 0.17 and 3.99 ± 0.45 L/min, respectively, for 50, 100 and 250 mg/kg oral doses. The significant difference in Cl/F values is due to the changes in V_1/F . If we multiply the Cl/F values by the corresponding F values, the clearance rates of scopoletin after oral administration were 0.374 ± 0.001 , 0.374 ± 0.001 and 0.374 ± 0.001 L/min, respectively. The values of clearance after oral administrations were not significantly different from the clearance (0.382 ± 0.057 L/min) after IV administration of 10 mg/kg. The mean volume of distribution of the central compartment divided by the bioavailability (V_1/F) were 126.86 ± 10.66 , 189.73 ± 13.11 and 219.29 ± 38.63 L, respectively. Again, if we also multiple the V_1/F values by the corresponding F values, we find the values of V_1 were 23.89 ± 1.16 , 21.56 ± 0.66 and 20.66 ± 3.14 L, respectively. Then, by using the relationship of $V_{ss} = V_1 (1 + k_{12}/k_{21})$, we find the V_{ss} values were 30.88 ± 0.52 , 28.43 ± 0.88 and 25.74 ± 1.44 L, re-

spectively. The V_{ss} of scopoletin is much higher after oral administration than after IV administration.

There were no statistically significant differences in clearance values among the three oral doses as estimated from Cl/F and bioavailability values. However, the k_{10} values increased as the dose increased as shown in Table 1. Based on the relationship of $Cl = k_{10} \cdot V_1$, the volume of distribution of the central compartment, V_1 , decreased as the dose increased as mentioned above.

In conclusion, the pharmacokinetics of scopoletin after oral administration are nonlinear over the dose range of 50-250 mg/kg in rabbits. The AUC values did not increase proportionally with increasing oral doses, and the values of K_{10} , V_1/F and Cl/F were increased as the dose was increased. The bioavailability of scopoletin after administration in rabbits was extremely low (0.10 to 0.19), likely due to the poor water solubility of scopoletin.

SYMBOLS

- C_p : concentration of drug in plasma
 A : intercept of distribution slope α with ordinate
 B : intercept of back-extrapolated monoexponential elimination slope β with ordinate
 k_a : absorption rate constant
 α : distribution rate constant
 β : elimination rate constant
 k_{10} : elimination rate constant from central compartment
 k_{12} : distribution rate constant for transfer of drug from central to peripheral compartment
 k_{21} : distribution rate constant for transfer of drug from peripheral to central compartment
AUC: Area under the plasma level-time curve
 k_a -HL: absorption half-life
 $t_{1/2\alpha}$: distribution half-life
 $t_{1/2\beta}$: elimination half-life
 V_1/F : apparent volume of distribution of the central compartment divide by the bioavailability
 Cl/F : clearance divide by the bioavailability
 t_{max} : the time of peak concentration
 C_{max} : the peak concentration

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